Eurachem 2014
QUALITY IN ANALYTICAL MEASUREMENTS

Validation of the Quantification of Cathinones In *plant feeders* by ^{1}H qNMR

Rudi Lopes, Maria J. Villa Brito, Helena Gaspar* and Ricardo Bettencourt da Silva

*Departamento de Química e Bioquímica, FCUL, Campo Grande, 1749-016 Lisboa, Portugal; *email -* **hmgaspar@fc.ul.pt**

INTRODUCTION

Cathinone, the main active principle of the plant *Catha edulis*, has been used as a prototype for the development of several synthetic derivatives that quickly came to market of legal drugs. These *designer drugs* are intentionally marketed as alternatives to illegal ones, aiming to circumvent drug legislation. The astonishing rate, at which new *designer drugs* appear, makes their impact on user's health unpredictable. Therefore it is extremely important to know the composition of these products. Those cathinones are normally sold in Europe as *plant feeders*. Previously we identified, by GC-MS and NMR, 19 psychoactive compounds in 27 *plant feeders* which showed hepatotoxic effects.[1]

This work reports the validation of the quantification of various cathinones in several synthetic drugs by ^{1}H qNMR using maleic acid as internal standard. The measurement procedures were subsequently applied to the characterisation of several *plant feeders* purchased in Portuguese *smart shops*.

The validation of these quantifications involved studying the performance of all analytical steps individually, the determination of the uncertainty of the reference value and the combination of these information using the uncertainty propagation.[2,3] The linearity assumption of the uncertainty propagation law was tested by the differences of uncertainty estimated by the numerical Kragten method using either positive or negative increments.[3,4]

The uncertainty associated with gravimetric and volumetric steps was estimated as suggested by the Eurachem/CITAC guide.[3] Models of the repeatability of the NMR signals were used to estimate the variability of the instrumental signal. The linearity of the NMR response was tested by the metrological compatibility[5] of the results using signals at different chemical shifts.

Measurement procedure

Weighing 10 mg of each plant feeder, dissolution with a 500µL standard solution of maleic acid (10 mg in 10 mL D_2O), sample solution dilution if required, and quantification by ^{1}H NMR.

qNMR parameters

400MHz Bruker NMR spectometer; size of fid (48000 data points); number of scans (16); delay (35s); spectral width (12 ppm); digital resolution (10 points/Hz); pulse program (zg, 90º pulse length); chemical shifts were referenced to maleic acid signal (6.42 ppm).

Measurement equation

$$W_{CAT} = \frac{I_{CAT}}{I_{MA}} \times \frac{n^H_{MA}}{n^H_{CAT}} \times \frac{V_2}{V_1} \times \frac{M_{CAT}}{M_{MA}} \times \frac{m^W_{MA}}{m^W_{SAMPLE}} \times P_{MA}$$

$\mathbf{W_{CAT}}$: mass fraction; $\mathbf{I_{CAT}}$: cathinone ^{1}H NMR peak integration; $\mathbf{I_{MA}}$: maleic acid ^{1}H NMR peak integration; $\mathbf{n^H_{MA}}$: number of maleic acid protons; $\mathbf{n^H_{CAT}}$: number of cathinone protons; $\mathbf{V_2}$: total standard solution volume ; $\mathbf{V_1}$: standard solution volume added to 10 mg of each cathinone; $\mathbf{M_{CAT}}$: cathinone molecular weight; $\mathbf{M_{MA}}$: maleic acid molecular weight; $\mathbf{m^W_{MA}}$: maleic acid weighed mass; $\mathbf{m^W_{SAMPLE}}$: cathinone weighed mass; $\mathbf{P_{MA}}$: maleic acid purity.

Measurement performance assessment

Analysis of plant feeders (Blast, Bliss and Blow), after various sample solution dilutions to study ^{1}H NMR signals at 3 different concentrations (1 mg, 2 mg, and 10 mg).

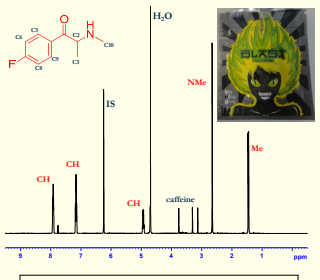
Measurement uncertainty evaluation

Instrumental signal precision was estimated from the repeatability of the ratio of analyte and internal standard signals. Models of the variation of this precision with signal intensity will be developed. The uncertainty with gravimetric and volumetric steps, and associated with molar masses, was estimated as described in the Eurachem/CITAC guide.

Uncertainty components were combined using the Kragten method after proving function linearity.

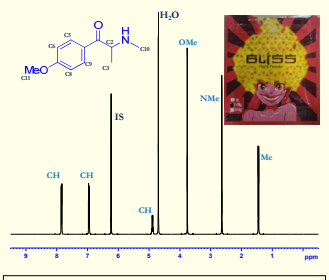
The proportionality of NMR signal from different chemical shifts was assessed taking signal repeatability into account.

RESULTS



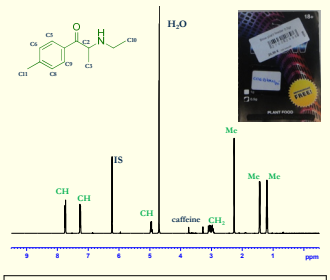
Flephedrone

Position	C3	C10	C2	C6-C8	C5-C9
δ_H, m	1.63d	2.83s	5.11q	7.33t	8.09dd
Weight (mg)	W_{CAT}	u	W_{CAT}	u	W_{CAT}	u	W_{CAT}	u	W_{CAT}	u
1	87.54%	1.55%	87.60%	1.55%	99.21%	8.88%	88.64%	1.26%	88.76%	1.26%
2	89.29%	0.99%	88.31%	0.89%	89.80%	7.45%	88.94%	0.92%	88.87%	0.92%
10	90.38%	1.63%	90.37%	1.63%	89.57%	1.82%	90.14%	1.63%	90.17%	1.63%



Methedrone

Position	C3	C10	C11	C2	C6-C8	C5-C9
δ_H, m	1.63d	2.82s	3.94s	5.07q	7.14d	8.03d
Weight (mg)	W_{CAT}	u	W_{CAT}	u	W_{CAT}	u	W_{CAT}	u	W_{CAT}	u	W_{CAT}	u
1	96.78%	3.81%	95.47%	3.59%	95.43%	5.66%	99.57%	8.72%	96.54%	3.57%	96.64%	3.58%
2	97.43%	7.63%	96.07%	7.50%	95.89%	7.49%	98.52%	7.92%	96.48%	7.52%	96.61%	7.53%
10	97.27%	1.82%	97.28%	1.82%	96.63%	1.80%	96.56%	1.83%	96.68%	1.81%	96.82%	1.81%



4-MEC

Position	C10	C3	C11	C2	C6-C8	C5-C9
δ_H, m	1.39t	1.63d	2.45s	5.15q	7.45d	7.94d
Weight (mg)	W_{CAT}	u	W_{CAT}	u	W_{CAT}	u	W_{CAT}	u	W_{CAT}	u	W_{CAT}	u
1	91.61%	3.76%	91.74%	3.74%	91.49%	3.61%	96.71%	6.42%	92.28%	3.59%	93.69%	3.69%
2	91.15%	2.11%	91.41%	2.12%	91.23%	2.10%	94.13%	2.45%	92.28%	2.17%	93.69%	2.28%
10	91.32%	1.61%	91.41%	1.60%	91.33%	1.59%	92.68%	1.65%	92.28%	1.61%	93.69%	1.64%

Based on ^{1}H qNMR analysis and the uncertainty evalution, the best signals to quantify the various compounds are: C10 for flephedrone, C11 for methedrone, and C11 for 4-MEC (4-methylethcathinone). The target relative standard uncertainty[5] of these determinations is 2.8% since differences in the composition of products of more than 10% are required to be distinguished[6].

References: (**1**) A. B. M. Araújo, M. J. Valente, M. Carvalho, D. D. Silva, H. Gaspar, F. Carvalho, M. L. Bastos, P. G. Pinho, *Archives Toxicology* (2014), submitted; (**2**) Joint Committee for Guides in Metrology, *Guide to the expression of uncertainty in measurement*, JCGM 100, BIPM, 2008; (**3**) Williams, S. L. R. Ellison (Eds), *Quantifying Uncertainty in Analytical Measurement*, 3rd Ed., Eurachem/CITAC, 2012; (**4**) J. Kragten, *Analyst* 119 **(1994)** 2161–2166; (**5**) Joint Committee for Guides in Metrology, *International vocabulary of metrology — basic and general concepts and associated terms (VIM)*, JCGM 200, BIPM, 2008; (**6**) R. J. N. Bettencourt da Silva, *Water* 5 **(2013)** 1279-1302.

Acknowledgements

This work was supported by national funding through FCT- Fundação para a Ciência e a Tecnologia (PEst-OE/QUI/UI0612/2014).